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Preparation of Selectively Deuterated d-γ-Tocotrienol

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Summary

The naturally occurring hypocholesterolemic and antioxidant agent d-γ-tocotrienol ((2*R*), 3'-*trans*, 7'-*trans*-tocotrienol) differs from the related tocopherols (Vitamin E) by possessing isoprenoid side-chain unsaturation. The presence of this unsaturation severely limits the chemistry available for tagging the substance. We were able to synthesize d-γ-tocotrienol-5-^{2}H from the natural product by selective bromination (NBS in CS_2) followed by transfer reduction using hydrazine-d_4 catalysed by Pd/C.

Key Words: tocotrienol, tocol, tocopherol, bromination, reduction, deuterium

Introduction

The vitamin E family of compounds (1) includes, in addition to the well-known tocopherols, several tocotrienols which bear an unsaturated isoprenoid side chain. One of these substances, d-γ-tocotrienol 1 ((2*R*), 3'-*trans*, 7'-*trans*-tocotrienol) has been found to possess hypocholesterolemic (2-4), antioxidant (5), and antitumor (6) activities.

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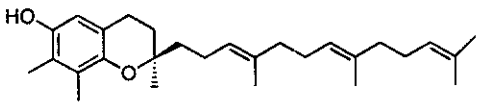
Since 1 is found at very low levels in common human diets (7), studies of the compound's bioavailability and metabolic fate would be best carried out using a deuterium-labeled sample. This technique has been successfully employed in studies of vitamin E bioavailability (8).



1

Vitamin E (d-α-tocopherol) has been labeled with ^{14}C, ^{13}C, and ^{2}H through attachment of a tagged methyl group to the aromatic ring of a less-highly methylated tocopherol (9,10,11) and with ^{2}H elsewhere in the molecule during total synthesis (11). The unavailability of significant quantities of the required monomethyl tocotrienol from natural sources and the difficulties inherent in total synthesis of the d-tocotrienols (2,12) prompted us to depart from techniques applied to Vitamin E and investigate instead the deuterium labelling of natural-source d-γ-tocotrienol as a route to our synthetic target.

Results and Discussion

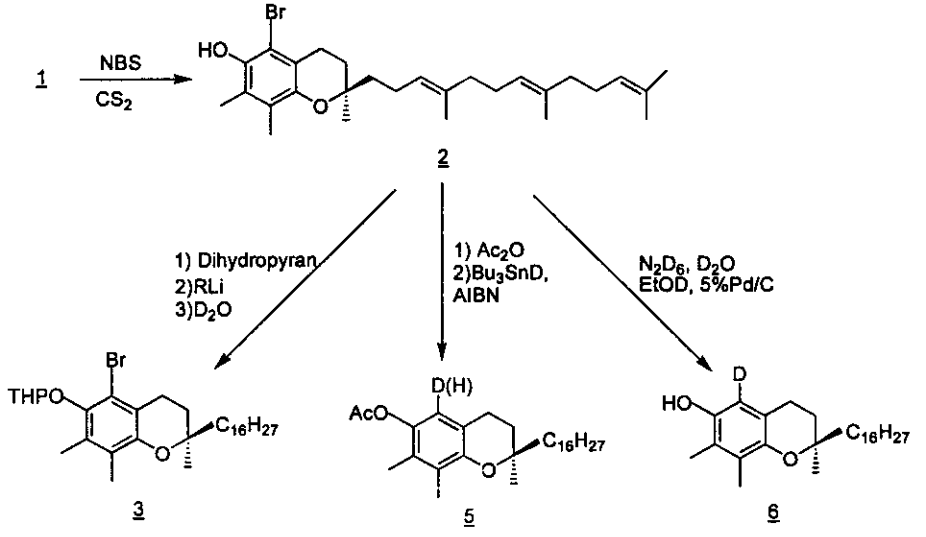
Although we briefly considered the possibility of cleaving (through ozonolysis) and reattaching (with Wittig chemistry) a tagged portion of the isoprenoid side chain of 1, the single unsubstituted aromatic site at position 5 of the structure beckoned as the easiest place to insert a label.

We initially examined simple exchange reactions on 1. Although base-catalysed exchange with D_2O gave no deuterium incorporation, treatment with about 20% D_2SO_4 in MeOD gave up to 75% deuterium incorporation at the 5-position. Unfortunately ^{13}C nmr spectroscopy revealed that this reaction was accompanied by considerable scrambling of the double bond geometry in the side chain.

Likewise disappointing were attempts to directly lithiate 1 or a derivative. Thus treatment of the tetrahydropyranyl ether of 1 with n- or sec-butyllithium under various conditions, followed by quenching with D_2O, gave no labeling at all.

We therefore turned our attention to producing the 5-bromo derivative of 1. After trying many reagents our best success came by reacting 1 with a slight excess of N-bromosuccinimide (NBS) in CS_2 at room temperature in the dark (13). This reaction gave 2 in 55% yield after chromatography. Protection of 2 as the tetrahydropyranyl ether 3, followed by attempted lithiation with n- or sec-butyllithium and D_2O quenching always returned unreacted bromoether 3. It would appear that the steric requirements for halogen-lithium exchange cannot be met in the hexasubstituted benzene 3. Similarly disappointing was an attempt to reduce the acetate 4 with tributyltin deuteride. Reaction of 4 with 98% Bu_3SnD in benzene (AIBN catalyst) gave γ-tocotrienyl acetate 5 which carried only about 60% deuterium at position 5. Although we lack a full understanding of the source of the protons that caused this failure, it seems possible that proton abstraction from the acetyl protecting group occurred.

Fortunately we were able to achieve a reasonably good reduction of bromophenol 2 using hydrogen-transfer conditions. Thus reaction of 2 with excess hydrazine-d_4 in D_2O/EtOD in the presence of 5% Pd/C catalyst gave, after chromatography, a 60% yield of labeled tocotrienol 6. The product contained 90-95% D (mass spec, nmr analyses) and there was no evidence for over-reduction or isomerization at the side chain olefins (^{13}C nmr analysis). Unreacted 2 could be recovered for recycle in about 25-30% yield from the reduction; thus the mass balance was 80% accountable.



Experimental

General: d-γ-Tocotrienol 1 was obtained in 92-96% purity from a commercial rice bran oil concentrate by a combination of countercurrent liquid-liquid extraction, silica gel chromatography, acetylation, further chromatography, and deacetylation. Hydrazine-d_4 deuterate (98 atom % D) was obtained from Isotec. Solvents and reagents were used as obtained. NMR spectra were obtained on a Varian Gemini 300 instrument in $CDCl_3$. Mass spectra were taken with a VG 7070SEQ instrument.

5-Bromo-d-γ-tocotrienol, 2: A solution of 1.28 grams (3.12mmol) of tocotrienol 1 in 15 ml of carbon disulfide was stirred under argon with protection from light. N-Bromosuccinimide (0.70g, 4mmol) was added in portions over 15 minutes and the reaction allowed to continue for 1.5 hrs. TLC analysis of the reaction at this point disclosed the consumption of most of the starting tocotrienol and formation of a single, less polar product and some tarry baseline material. Most of the solvent was removed in a stream of argon and the residue dissolved in ethyl acetate. The resulting solution was washed with water, dried over $MgSO_4$, stripped of solvent on the rotovap, and the residue flash-chromatographed on silica gel with 1% acetone in hexane elution. The non-polar product fraction afforded 0.84 gram (55% yield) of 2 as a pale yellow syrup. The substance was highly susceptible to air oxidation and was generally used as soon as possible. NMR: δ 5.10 (sharp s, 1H, phenol), 5.03-5.08 (m, 3H, vinyl), 2.66 (br t, 2H, 4-position CH_2), 2.22 (s, 3H, Me), 2.11 (s, 3H, Me), 1.26 (s, 3H, 2-Me), 1.4-2.1 (complex m, 26H). FD Mass Spectrum: m/e 488, 1Br (calc for $C_{28}H_{41}{}^{79}BrO_2$ 488).

d-γ-Tocotrienol-5-D, 6: A solution of 2.23 g (4.57 mmol) of bromide 2 in 20 ml of 98 atom % EtOD was stirred at room temperature and purged with argon for 30 minutes.
There was then added 0.10 gram of 5% Pd/C catalyst, followed by dropwise addition of 0.50 grams of hydrazine-d_4 deuterate (98 atom % d). The mixture was stirred under reflux for 1.5 hrs, at which time TLC analysis indicated about 25% conversion of 2 to a compound having the rf of tocotrienol 1. The product composition did not change after a further hour's reflux, so an additional 0.10 gram of catalyst and 0.5 gram of hydrazine was added and the mixture refluxed a further hour. At this

time a third addition of catalyst and hydrazine was made and reflux continued 2 more hours. When it appeared that no further conversion to product was occurring, the reaction was cooled, filtered (celite), drowned into water, and extracted with ether. The organic phase was washed with water, 5% HCl, 5% $NaHCO_3$, brine, and dried and stripped of solvent on the rotovap. The resulting syrup was chromatographed on silica gel (acetone-hexane gradient, 1-5%) to give 0.62 g (27%) recovered bromide 2 and 1.14 g (60%) of labeled tocotrienol 6 as a yellow syrup. NMR: δ 6.38 (s, 0.07 H, residual 5-H), 5.12 (m, 3H, vinyls), 2.66 (br t, 2H, 4-CH_2), 2.16 (s, 3H, Me), 2.13 (s, 3H, Me), 1.25 (s, 3H, 2-Me); 1.2-2.1 (complex m, 26H). FD Mass spectrum: m/e 411 (calc for $C_{28}H_{41}DO_2$ 411).

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